

# Preparation of 1,8-Di-*O*-alkylaloe-emodins and 15-Amino-, 15-Thiocyano-, and 15-Selenocyanochrysophanol Derivatives from Aloe-Emodin and Studying Their Cytotoxic Effects

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**1,8-Di-*O*-alkylaloe-emodin derivatives** (namely, methyl-, propyl-, hexyl-, dodecyl-, and octadecyl-) were synthesized from naturally occurring aloe-emodin. Further, derivatives having various substituents such as diethyl-amino, pyrrolidinyl, piperidinyl, methylpiperazinyl, imidazolyl, thiocyno and selenocyno groups at the 15 position of chrysophanol and 1,8-di-*O*-hexylchrysophanol from aloe-emodin were synthesized. The cytotoxic effects of these derivatives on less P-glycoprotein (P-gp)-expressing HCT 116 cells and stably P-gp-expressing Hep G2 cells were evaluated by performing 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Among these products, several of them exhibited markedly higher potent cytotoxic effects not only on HCT116 cells but also Hep G2 cancer cells as compared to aloe-emodin.

**Key words** 1,8-di-*O*-alkylaloe-emodin; 15-aminochrysophanol; 15-thiocyanochrysophanol; 15-selenocyanochrysophanol; cytotoxic effect

Previously, we reported that when the cytotoxic effects of 4'-*O*-alkylaloeenin derivatives (from methyl to hexadecyl) derived from aloenin (**1**) are compared, the longer the alkyl chain of the 4'-*O*-alkylaloeenin derivatives, the greater is the enhancement in the cytotoxic effects on human colorectal (HCT 116) and human hepatoma (Hep G2) cancer cell lines.<sup>1)</sup> Furthermore, we reported that pseudodiosgenone derivatives having various amino groups, thiocyno (SCN), and selenocyno (SeCN) groups at the 26 position derived from diosgenin (**2**) exhibited the potent cytotoxic activities against the same cancer cell lines,<sup>2)</sup> while compounds **1** and **2** have not been known to exhibit cytotoxic activity yet. In our preliminary investigation, these compounds also showed no marked cytotoxicity in less than 500  $\mu$ M of the IC<sub>50</sub> value—the concentration at which 50% of the cells are inhibited from growing. However, from the both above-mentioned reports, it is evident that a compound that possessed an alkyl chain with an appropriate length or that introduced appropriate substituents such as amino, thiocyno, and selenocyno groups in the molecule seemed to exhibit potent cytotoxic activity.

In the present study, we investigate the preparation of 1,8-di-*O*-alkylaloe-emodins and 15-amino-, 15-thiocyano-, and 15-selenocyanochrysophanol derivatives from aloe-emodin (**3**) which has been known to have several pharmacological activities such as cytotoxic activity,<sup>3–5)</sup> antifungal activity,<sup>6)</sup> antibacterial activity,<sup>7)</sup> and so on, and further we evaluate the cytotoxic effects of the synthetic derivatives on HCT 116 and Hep G2 cancer cell lines.

## Results and Discussion

Aloe-emodin **3** is an anthraquinone derivative isolated from many Chinese herbal medicines such as *Aloe arborenses* MILL. var. *natalensis* BERGER (Liliaceae),<sup>8,9)</sup> *Rheum palumatum* L. (Polygonaceae),<sup>10,11)</sup> *Cassia tora* L. (Leguminosae),<sup>12)</sup> and so on. This anthraquinone derivative has two

phenolic hydroxyl groups at the 1 and 8 positions and one aliphatic hydroxyl group at the 15 position. In this study, the alkylations of the phenolic hydroxyl groups and the substitution reactions with various amino groups and thiocyno and selenocyno groups at the 15 position of **3** were performed to compare their cytotoxic activities.

First, several alkyl derivatives were prepared from **3**. The methylation of **3** with dimethylsulfate in the presence of K<sub>2</sub>CO<sub>3</sub> was done according to the method reported by Ross and Mitali.<sup>13)</sup> to give 1,8-di-*O*-methylaloe-emodin **4** in a 61.6% yield. Other alkylations were performed according to the method of MacTough *et al.*<sup>14)</sup> Compound **3** was reacted with propyl iodide, hexyl bromide, dodecyl iodide, and octadecyl iodide in the presence of K<sub>2</sub>CO<sub>3</sub> to give 1,8-di-*O*-propyl- **5**, 1,8-di-*O*-hexyl- **6**, 1,8-di-*O*-dodecyl- **7**, and 1,8-di-*O*-octadecylaloe-emodin **8** in 14.5, 49.0, 40.0 and 36.0%

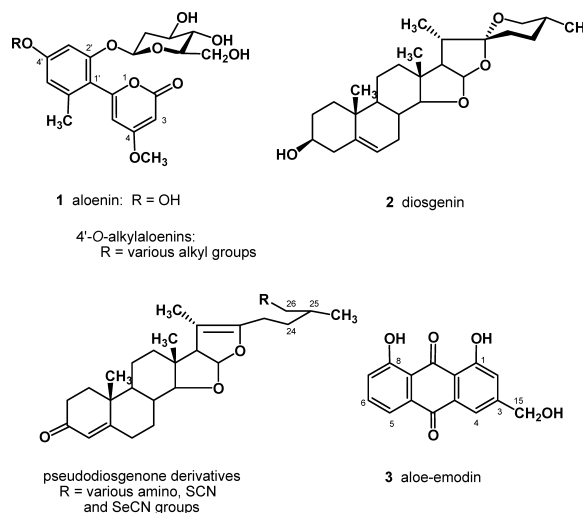
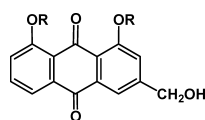


Fig. 1. Structures of Aloenin **1**, 4'-*O*-Alkylaloeenins, Diosgenin **2**, Pseudodiosgenone Derivatives and Aloe-Emodin **3**

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- 4 R = CH<sub>3</sub>  
 5 R = CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>  
 6 R = CH<sub>2</sub>(CH<sub>2</sub>)<sub>4</sub>CH<sub>3</sub>  
 7 R = CH<sub>2</sub>(CH<sub>2</sub>)<sub>10</sub>CH<sub>3</sub>  
 8 R = CH<sub>2</sub>(CH<sub>2</sub>)<sub>16</sub>CH<sub>3</sub>

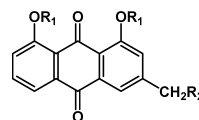
Fig. 2. Structures of 1,8-Di-*O*-alkylaloe-emodins 4—8Table 1. Comparison of Cytotoxic Effects of Various 1,8-*O*-Dialkylaloe-emodin Derivatives 4—8 with Aloe-Emodin 3

Compound	IC <sub>50</sub> ± S.D. (μM) <sup>a)</sup>	
	HCT 116 cells	Hep G2 cells
3	8.7 ± 0.8	10.0 ± 0.9
4	40.8 ± 5.7	>100 <sup>b)</sup>
5	46.9 ± 4.3	>100 <sup>b)</sup>
6	9.7 ± 3.5	>100 <sup>b)</sup>
7	77.9 ± 2.8 <sup>c)</sup>	>100 <sup>b,c)</sup>
8	76.3 ± 2.5 <sup>c)</sup>	>100 <sup>b,c)</sup>

<sup>a)</sup> IC<sub>50</sub> values (mean ± S.D.) are the concentrations at which 50% of the cells are inhibited from growing. <sup>b)</sup> IC<sub>50</sub> values more than 100 μM are indicated as >100. <sup>c)</sup> Exact values of these compounds were not obtained because of the turbidity in the concentrations as indicated by the IC<sub>50</sub> values.

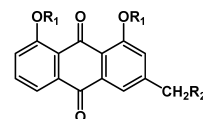
yields, respectively.

The cytotoxic effects of alkylaloe-emodin derivatives were tested by performing the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay<sup>15)</sup> using human colorectal (HCT 116)<sup>16)</sup> and human hepatoma (Hep G2)<sup>17)</sup> cancer cell lines, and the effects were represented by IC<sub>50</sub> values—the concentrations at which 50% of the cells are inhibited from growing. The former expresses very little MDR 1 (P-glycoprotein; P-gp), while the latter overexpresses P-gp.<sup>17)</sup> It has been known that P-gp acts as an efflux pump to remove several antitumor agents such as Ca<sup>2+</sup> antagonist, cyclosporine, and dioxin from P-gp-overexpressing cells.<sup>18)</sup> Among alkylaloe-emodins 4—8, only 1,8-di-*O*-hexylaloe-emodin 6 showed an almost similar effect (IC<sub>50</sub> value: 9.7 ± 3.5 μM) on HCT 116 cells as compared to that of aloe-emodin 3 (IC<sub>50</sub> value: 8.7 ± 0.8 μM) (Table 1). The activities of 1,8-*O*-dimethyl- 4 and 1,8-*O*-dipropylaloe-emodin 5 (IC<sub>50</sub> values: 40.8 ± 5.7 and 46.9 ± 4.3 μM, respectively), which had shorter alkyl chains than that of 6, decreased. These results suggest that there is no relationship between the cytotoxic activity and the alkyl chain lengths of alkylaloe-emodin derivatives against HCT 116 cells. Although it was difficult to determine the exact IC<sub>50</sub> values for 1,8-di-*O*-dodecyl- 7 and 1,8-di-*O*-octadecylaloe-emodin 8 (because of the turbidity in the concentrations that were used to determine the IC<sub>50</sub> value), they showed no marked activities. In the case of Hep G2 cells, only 3 showed potent activity (IC<sub>50</sub> value: 10.0 ± 0.9 μM) which was almost similar to the value of 3 in the case of HCT 116 cells, while all alkylated aloe-emodins 4—8 showed no marked activity. This result indicates that aloe-emodin derivatives introducing alkyl groups at the 1 and 8 positions may be influenced by the efflux pump of P-gp in



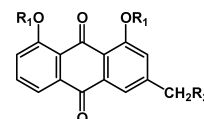
- 9 R<sub>1</sub> = H, R<sub>2</sub> = Br  
 15 R<sub>1</sub> = hexyl, R<sub>2</sub> = I  
 16 R<sub>1</sub> = hexyl, R<sub>2</sub> = OTs  
 (Ts = *p*-toluenesulfonyl)  
 24 R<sub>1</sub> = hexyl, R<sub>2</sub> = Br

Fig. 3. Structures of Compounds 9, 15, 16 and 24



- 10 R<sub>1</sub> = H, R<sub>2</sub> = —N(C<sub>2</sub>H<sub>5</sub>)<sub>2</sub>  
 11 R<sub>1</sub> = H, R<sub>2</sub> = —N(CH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>  
 12 R<sub>1</sub> = H, R<sub>2</sub> = —N(CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>  
 13 R<sub>1</sub> = H, R<sub>2</sub> = —N(CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>  
 14 R<sub>1</sub> = H, R<sub>2</sub> = —N(CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>  
 17 R<sub>1</sub> = hexyl, R<sub>2</sub> = —N(C<sub>2</sub>H<sub>5</sub>)<sub>2</sub>  
 18 R<sub>1</sub> = hexyl, R<sub>2</sub> = —N(CH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>  
 19 R<sub>1</sub> = hexyl, R<sub>2</sub> = —N(CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>  
 20 R<sub>1</sub> = hexyl, R<sub>2</sub> = —N(CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>  
 21 R<sub>1</sub> = hexyl, R<sub>2</sub> = —N(CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>

Fig. 4. Structures of Compounds 10—14 and 17—21



- 22 R<sub>1</sub> = H, R<sub>2</sub> = —SCN  
 23 R<sub>1</sub> = H, R<sub>2</sub> = —SeCN  
 25 R<sub>1</sub> = hexyl, R<sub>2</sub> = —SCN  
 26 R<sub>1</sub> = hexyl, R<sub>2</sub> = —SeCN

Fig. 5. Structures of Compounds 22, 23, 25 and 26

Hep G2 cells.

Next, the hydroxyl group at the 15 position of aloe-emodin 3 and 1,8-di-*O*-hexylaloe-emodin 6, which showed potent cytotoxic activities against HCT 116 cells, was replaced with amino groups and thiocyno (SCN) and selenocyno (SeCN) groups, and the cytotoxic activities of the products were further evaluated. The treatment of 3 with CBr<sub>4</sub> and PPh<sub>3</sub> in tetrahydrofuran (THF) gave bromide 9 in 81.9% yields, which was used as the starting material for the introduction of various amino groups at the 15 position of 3. Compound 9 was reacted with diethylamine, pyrrolidine, piperidine, 1-methylpiperazine and imidazole according to the method of Quan *et al.*<sup>19)</sup> to give compounds 10—14 (in 48.3, 58.0, 28.6, 33.7, 19.3% yields, respectively). In the preparation of amino derivatives from 1,8-di-*O*-hexylaloe-emodin 6, iodide 15 was used as the starting material in order to increase the yield. Compound 15 was derived from 6 as follows: the tosylation<sup>20)</sup> of 6 gave tosylate 16 that reacted with sodium iodide to give 15. The reaction of 15 with diethylamine, pyrrolidine, piperidine, 1-methylpiperazine, and imidazole gave 17—21 (in 58.0, 62.0, 52.0, 48.3, 66.5% yields, respectively).

Next, the hydroxyl group at the 15 position of compounds 3 and 6 was replaced with thiocyno (SCN) and selenocyno (SeCN) groups. The reaction of bromide 9 with KSCN and

Table 2. Cytotoxic Effects of 15-Aminochrysophanols **10**–**14** and 1,8-Di-*O*-hexyl-15-aminochrysophanols **17**–**21**

Compound	IC <sub>50</sub> ± S.D. (μM) <sup>a)</sup>	
	HCT 116 cells	Hep G2 cells
<b>10</b>	1.9 ± 0.2	2.1 ± 0.5
<b>11</b>	3.6 ± 0.6	3.5 ± 0.7
<b>12</b>	3.7 ± 0.9	4.4 ± 0.6
<b>13</b>	2.5 ± 0.1	2.3 ± 0.4
<b>14</b>	2.5 ± 0.3	2.0 ± 0.2
<b>17</b>	3.0 ± 0.6	5.3 ± 0.7
<b>18</b>	10.2 ± 0.9	28.3 ± 3.6
<b>19</b>	9.2 ± 0.4	33.1 ± 3.6
<b>20</b>	2.4 ± 0.2	2.2 ± 0.3
<b>21</b>	5.3 ± 0.6	8.4 ± 0.9

a) IC<sub>50</sub> values (mean ± S.D.) are the concentrations at which 50% of the cells are inhibited from growing.

KSeCN gave 15-thiocyanochrysophanol **22** and 15-selenochrysophanol **23** in 53.5 and 65.1% yields, respectively. Similarly, bromide **24** derived from **6** by bromination was treated with KSCN and KSeCN to give 1,8-di-*O*-hexyl-15-thiocyanochrysophanol **25** and 1,8-di-*O*-hexyl-15-selenochrysophanol **26** in 76.6 and 86.2% yields, respectively.

Koyama *et al.*<sup>21)</sup> already reported the cytotoxic effects of 15-aminochrysophanol derivatives **10**, **11**, **12** and **14** using murine L1210 leukemic cells human acute promyelocytic leukemia cells (HL-60). In this study, we investigated the cytotoxic effects of 15-aminochrysophanol derivative **13** and 15-amino-1,8-di-*O*-hexylchrysophanol derivatives **17**–**21** together with **10**, **11**, **12** and **14** against HCT 116 and Hep G2 cells; the results are listed in Table 2. All 15-aminochrysophanol derivatives **10**–**14** exhibited higher cytotoxic effects (IC<sub>50</sub> values: 1.9 ± 0.2 to 3.7 ± 0.9 μM) than that of **3** (IC<sub>50</sub> value: 8.7 ± 0.8 μM) on HCT 116 cells. Further, these compounds showed higher effects (IC<sub>50</sub> values: 2.0 ± 0.2 to 4.4 ± 0.6 μM) than that of **3** (IC<sub>50</sub> value: 10.0 ± 0.9 μM) on Hep G2 cells. Thus, the replacement of the hydroxyl group at the 15 position of **3** with amino groups enhanced the cytotoxic effect. In the case of 1,8-di-*O*-hexyl-15-aminochrysophanol derivatives **17**–**21**, 15-diethylamino, 4-methylpiperazin-1-yl, and imidazol-1-yl derivatives (**17**, **20**, **21**, respectively) enhanced the cytotoxic effects (IC<sub>50</sub> value: 2.4 ± 0.2 to 5.3 ± 0.6 μM) on HCT 116 cells, although the effects of pyrrolidin-1-yl **18** (IC<sub>50</sub> value: 10.2 ± 0.9 μM) and piperidin-1-yl derivative **19** (IC<sub>50</sub> value: 9.2 ± 0.4 μM) on the same cells were almost similar to that of **6** (IC<sub>50</sub> value: 9.7 ± 3.5 μM). In the case of Hep G2 cells, compounds **17**–**21** showed considerably higher potent effects than **6** (IC<sub>50</sub> value: more than 100 μM); in particular, **17**, **20**, and **21** exhibited marked potent effects (IC<sub>50</sub> values: 5.3 ± 0.7, 2.2 ± 0.3, 8.4 ± 0.9 μM, respectively). In this case, the effects of 15-(pyrrolidin-1-yl) **18** and 15-(piperidin-1-yl) derivative **19** were lower than those of **17**, **20**, and **21**. This result suggests that compounds **18** and **19** seem to be more sensitive to P-gp of Hep G2 cells than **17**, **20**, and **21**.

The cytotoxic effects of 15-thiocyano- and 15-selenochrysophanol derivatives, **22** and **23**, and 1,8-di-*O*-hexyl-15-thiocyano- and 1,8-di-*O*-hexyl-15-selenochrysophanol derivatives, **25** and **26**, derived from

Table 3. Cytotoxic Effects of **22**, **23**, **25** and **26** on HCT 116 and Hep G2 Cells

Compound	IC <sub>50</sub> ± S.D. (μM) <sup>a)</sup>	
	HCT 116 cells	Hep G2 cells
<b>22</b>	2.8 ± 0.5	22.5 ± 3.1
<b>23</b>	3.0 ± 0.6	5.9 ± 0.9
<b>25</b>	1.9 ± 0.3	7.6 ± 0.8
<b>26</b>	0.2 ± 0.08	0.7 ± 0.1

a) IC<sub>50</sub> values (mean ± S.D.) are the concentrations at which 50% of the cancer cells are inhibited from growing.

aloe-emodin **3** and 1,8-di-*O*-hexylaloe-emodin **6**, respectively, were also investigated (Table 3). All these derivatives enhanced the cytotoxic effects on HCT 116 cells as well as on Hep G2 cells more than **3** and **6**. 15-Thiocyanochrysophanol **22** and 15-selenochrysophanol **23** exhibited markedly potent effects (IC<sub>50</sub> values: 2.8 ± 0.5, 3.0 ± 0.6 μM on HCT 116 cells and 22.5 ± 3.1, 5.9 ± 0.9 μM on Hep G2 cells, respectively). 1,8-Di-*O*-hexyl-15-thiocyanochrysophanol **25** and 1,8-di-*O*-hexyl-15-selenochrysophanol **26** had much more potent effects (IC<sub>50</sub> values: 1.9 ± 0.3, 0.2 ± 0.08 μM on HCT 116 cells and 7.6 ± 0.8, 0.7 ± 0.1 μM on Hep G2 cells, respectively) than **22** and **23**, respectively. Thus, the introduction of SCN and SeCN groups into the aloe-emodin molecules enhanced the cytotoxic effect.

## Conclusions

In this study, 1,8-di-*O*-alkylaloe-emodins (**4**–**8**), 15-aminochrysophanols (**10**–**14**), 15-amino-1,8-di-*O*-hexylchrysophanols (**17**–**21**), 15-thiocyano- and 15-selenochrysophanols (**22**, **23**, respectively), and 1,8-di-*O*-hexyl-15-thicyano- and 1,8-di-*O*-hexyl-15-thicyanochrysophanols (**25**, **26**, respectively) were prepared from naturally occurring aloe-emodin **3**, and the cytotoxic effects of these compounds were evaluated using HCT 116 and Hep G2 cancer cell lines.

In alkyl derivatives **4**–**8**, only 1,8-di-*O*-hexylaloe-emodin **6** (IC<sub>50</sub> value: 9.7 ± 3.5 μM) showed a potent cytotoxic effect on HCT 116 cells similar to that of **3** (IC<sub>50</sub> value: 8.7 ± 0.8 μM). The effects of 15-aminochrysophanol **10**–**14** obtained by replacement of the hydroxyl group at the 15 position of **3** with diethylamino, pyrrolidinyl, piperidinyl, 4-methylpiperazinyl and imidazolyl, respectively, enhanced about 4.6–2.4-fold on HCT 116 cells. The replacement of the hydroxyl group at the 15 position of **6** with diethylamino, 4-methylpiperazinyl, and imidazolyl groups enhanced the effects on HCT 116 cells (IC<sub>50</sub> values: 2.4 ± 0.2 to 5.3 ± 0.6 μM), although the replacement of that with pyrrolidinyl and piperidinyl groups showed similar effects (IC<sub>50</sub> values: 10.2 ± 0.9, 9.2 ± 0.4 μM, respectively) to that of **3**. Furthermore, all amino derivatives **10**–**14** and **17**–**21** enhanced the effects on stably P-gp-expressing Hep G2 cells.

The replacement of the hydroxyl group at the 15 position of **3** and **6** with SCN and SeCN groups also enhanced the cytotoxic effects (Table 3). In the case of Hep G2 cells, selenochrysophanol derivatives **23** (IC<sub>50</sub> value: 5.9 ± 0.9 μM) and **26** (IC<sub>50</sub> value: 0.7 ± 0.1 μM) showed higher potent effect on stably P-gp-expressing Hep G2 cells than corresponding thiocyanochrysophanol derivatives **22** (IC<sub>50</sub> value: 22.5 ± 3.1 μM) and **25** (IC<sub>50</sub> value: 7.6 ± 0.8 μM), respectively.

In this study, we found that 15-aminochrysophanols **10**—**14**, 15-amino-1,8-di-*O*-hexylchrysophanols **17**, **20**, and **21**, 15-selenochrysophanol **23**, 1,8-di-*O*-hexyl-15-thiochrysophanol **25** and 1,8-di-*O*-hexyl-15-selenochrysophanol **26** exhibited markedly potent cytotoxic effects on stably P-gp-expressing Hep G2 cancer cells. Although the exact structure–activity relationship for the affinity to enzymes, intracellular accumulation, and so on has not been resolved yet, these derivatives may help to overcome drug excretion from stably P-gp-expressing cancer cells caused by multidrug resistance without any modulator.

## Experimental

**General Methods** Reagent-grade chemicals and solvents were obtained from commercial suppliers. The melting points (mp) were determined using a Yanagimoto micromelting point apparatus and were uncorrected. Kieselgel 60 F<sub>254</sub> (E. Merck) was used in thin-layer chromatography (TLC). Spots were detected by spraying plates with 1:9 Ce(SO<sub>4</sub>)<sub>2</sub>–10% H<sub>2</sub>SO<sub>4</sub> reagent, followed by heating the plates at 250 °C for 4–5 min. Preparative TLC was performed using silica gel 60 F<sub>254</sub> (Merck, 200×200×2 mm). Column chromatography was carried out using Kieselgel 60 (E. Merck), then the eluates were monitored by TLC. An SSC-6300 HPLC instrument (Senshu Co., Ltd.) was employed for analytical HPLC using column DOCOSIL (Senshu Co., Ltd.; 10×250 mm) attached to an SSC autoinjector 6310 and an SSC fraction collector 6320 for preparative HPLC. <sup>1</sup>H- and <sup>13</sup>C-NMR spectroscopy investigations were carried out at 500 and 125 MHz, respectively, and <sup>1</sup>H–<sup>1</sup>H and <sup>1</sup>H–<sup>13</sup>C COSY and HMBC spectra were obtained using a JEOL JNM-A500 FT-NMR spectrometer. Tetramethylsilane was used as an internal standard. The chemical shifts are given in ppm. The multiplicities of the <sup>1</sup>H-NMR signals are indicated as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), quin (quintet), and m (multiplet). Electron impact mass spectrum (EI-MS) and fast-atom-bombardment mass spectrum (FAB-MS) were recorded using a JEOL JMS-DX 300 mass spectrometer. The high-resolution mass (HR-MS) spectra were recorded using JEOL JMS-700.

**1,8-Di-*O*-methylaloe-emodin (4)** A solution of **3** (100 mg, 0.37 mmol) in dry acetone (10 ml) was added with dimethylsulfate (230 mg, 1.8 mmol) and absolute K<sub>2</sub>CO<sub>3</sub> (250 mg, 1.8 mmol), and then refluxed overnight. The reaction mixture was added with more dimethylsulfate (230 mg, 1.8 mmol) and absolute K<sub>2</sub>CO<sub>3</sub> (250 mg, 1.8 mmol) and refluxed for 12 h. The mixture was poured into ice-cold water (50 ml) and extracted with AcOEt (50 ml×3). The AcOEt extracts were successively washed with brine and water, dried over absolute Na<sub>2</sub>SO<sub>4</sub>, and then filtered. The filtrate was evaporated to give a residue that was subjected to column chromatography (a gradient of 0–5% AcOEt in hexane), followed by preparative HPLC (solvent system: 55% H<sub>2</sub>O in acetone, flow rate of 1.0 ml/min, and column temperature of 40 °C) to obtain **4** (68.2 mg, 61.6%) as yellow needles, mp 223–226 °C (after recrystallization from acetone). FAB-MS: *m/z* 299 [M+H]<sup>+</sup>. HR-MS (FAB): *m/z* Calcd for C<sub>17</sub>H<sub>15</sub>O<sub>5</sub> [M+H]<sup>+</sup>; 299.0919, Found; 299.0921. <sup>1</sup>H-NMR (*d*<sub>6</sub>-DMSO) δ: 7.74 (1H, t, *J*=8.2 Hz, H-6), 7.68 (1H, dd, *J*=8.2, 0.9 Hz, H-5), 7.67 (1H, brs, H-4), 7.53 (1H, dd, *J*=8.2, 0.9 Hz, H-7), 7.45 (1H, brs, H-2), 5.54 (1H, t, *J*=5.8 Hz, 15-CH<sub>2</sub>OH), 4.64 (2H, d, *J*=5.8 Hz, 15-CH<sub>2</sub>OH), 3.92 (6H, s, 1-, 8-OCH<sub>3</sub>). <sup>13</sup>C-NMR (CDCl<sub>3</sub>) δ: 183.4 (C-9), 181.1 (C-10), 158.8 (C-8), 158.7 (C-1), 149.7 (C-3), 134.1 (C-11), 134.1 (C-14), 133.9 (C-6), 123.5 (C-13), 122.0 (C-12), 118.9 (C-7), 118.1 (C-5), 115.9 (C-4), 115.5 (C-2), 62.3 (C-15), 56.3 (OCH<sub>3</sub>), 56.2 (OCH<sub>3</sub>).

**1,8-Di-*O*-propylaloe-emodin (5)** To a solution of **3** (100 mg, 0.37 mmol) in absolute DMF (10 ml), propyl iodide (460 mg, 2.7 mmol) and absolute K<sub>2</sub>CO<sub>3</sub> (260 mg, 1.9 mmol) were added, and then the mixture was refluxed overnight. The reaction mixture was extracted with AcOEt (50 ml×3). The organic extracts were successively washed with 5% HCl, 10% NaHCO<sub>3</sub> and water, dried over absolute MgSO<sub>4</sub>, and then filtered. The filtrates were evaporated to give a residue that was subjected to column chromatography (a gradient of 0–5% AcOEt in hexane), followed by preparative HPLC (column: DOCOSIL 10×250 mm, solvent: 50% H<sub>2</sub>O in acetone, flow rate: 1 ml/min, column temperature: 40 °C) to give **5** (19.0 mg, 14.5%) as yellow needles, mp 126–128 °C (after recrystallization from methanol). FAB-MS: *m/z* 355 [M+H]<sup>+</sup>. HR-MS (FAB): *m/z* Calcd for C<sub>21</sub>H<sub>23</sub>O<sub>5</sub> [M+H]<sup>+</sup>; 355.1565, Found; 355.1545. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 7.78 (1H, dd, *J*=7.6, 0.9 Hz, H-5), 7.66 (1H, brs, H-4), 7.57 (1H, t, *J*=7.6 Hz, H-6), 7.30 (1H, brs, H-2), 7.27 (1H, dd, *J*=7.6, 0.9 Hz, H-7), 4.77 (2H, s, 15-CH<sub>2</sub>OH),

4.10 and 4.06 (each 2H, t, *J*=6.7 Hz, 1- or 8-OCH<sub>3</sub>), 1.94 (4H, CH<sub>2</sub>×2), 1.13 and 1.11 (each 3H, t, *J*=7.3 Hz, CH<sub>3</sub>×2). <sup>13</sup>C-NMR (CDCl<sub>3</sub>) δ: 184.2 (C-9), 182.1 (C-10), 159.4 (C-8), 159.0 (C-1), 147.4 (C-3), 136.0 (C-14), 134.7 (C-11), 133.5 (C-6), 124.5 (C-13), 123.4 (C-12), 119.7 (C-7), 118.9 (C-5), 117.0 (C-4), 116.4 (C-2), 71.0 (OCH<sub>2</sub>×2), 64.4 (C-15), 22.5 (CH<sub>2</sub>×2), 14.1 (CH<sub>3</sub>×2).

**1,8-Di-*O*-hexylaloe-emodin (6)** The general alkylation of **3** (100 mg, 0.37 mmol) with hexyl bromide (310 mg, 1.9 mmol) gave **6** (79.6 mg, 49.0%) as yellow needles, mp 97–99 °C (after recrystallization from acetone). FAB-MS: *m/z* 439 [M+H]<sup>+</sup>. HR-MS (FAB): *m/z* Calcd for C<sub>27</sub>H<sub>35</sub>O<sub>5</sub> [M+H]<sup>+</sup>; 439.2484, Found; 439.2489. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 7.75 (1H, dd, *J*=7.6, 0.9 Hz, H-5), 7.59 (1H, d, *J*=1.5 Hz, H-4), 7.56 (1H, t, *J*=7.6 Hz, H-6), 7.26 (1H, d, *J*=1.5 Hz, H-2), 7.26 (1H, dd, *J*=7.6, 0.9 Hz, H-7), 4.73 (2H, s, 15-CH<sub>2</sub>OH), 4.12 and 4.06 (each 2H, t, *J*=6.7 Hz, 1- or 8-OCH<sub>2</sub>), 1.90 (4H, m, CH<sub>2</sub>×2), 1.55 (4H, m, CH<sub>2</sub>×2), 1.37 (8H, m, CH<sub>2</sub>×4), 0.92 (6H, m, CH<sub>3</sub>×2). <sup>13</sup>C-NMR (CDCl<sub>3</sub>) δ: 184.0 (C-9), 182.0 (C-10), 159.2 (C-8), 158.8 (C-1), 147.6 (C-3), 134.6 (C-14), 134.4 (C-11), 133.4 (C-6), 124.2 (C-13), 122.9 (C-12), 119.5 (C-7), 118.7 (C-5), 116.8 (C-4), 116.2 (C-2), 69.6 (OCH<sub>2</sub>×2), 64.2 (C-15), 31.4 (CH<sub>2</sub>×2), 29.0 (CH<sub>2</sub>), 28.9 (CH<sub>2</sub>), 25.5 (CH<sub>2</sub>×2), 22.5 (CH<sub>2</sub>×2), 13.9 (CH<sub>3</sub>×2).

**1,8-Di-*O*-dodecylaloe-emodin (7)** The general alkylation of **3** (100 mg, 0.37 mmol) with dodecyl iodide (550 mg, 1.9 mmol) gave **7** (90.0 mg, 40.0%) as yellow needles, mp 110–112 °C (after recrystallization from acetone). FAB-MS: *m/z* 607 [M+H]<sup>+</sup>. HR-MS (FAB): *m/z* Calcd for C<sub>39</sub>H<sub>59</sub>O<sub>5</sub> [M+H]<sup>+</sup>; 607.4363, Found; 607.4362. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 7.74 (1H, dd, *J*=7.6, 0.9 Hz, H-5), 7.57 (1H, brs, H-4), 7.56 (1H, t, *J*=7.6 Hz, H-6), 7.26 (1H, dd, *J*=7.6, 0.9 Hz, H-2), 7.25 (1H, brs, H-7), 4.73 (2H, s, 15-CH<sub>2</sub>OH), 4.12 and 4.04 (each 2H, t, *J*=6.7 Hz, 1- or 8-OCH<sub>2</sub>), 1.90 (4H, m, CH<sub>2</sub>×2), 1.53 (4H, m, CH<sub>2</sub>×2), 1.26 (32H, CH<sub>2</sub>×16), 0.88 (6H, t, *J*=6.7 Hz, CH<sub>3</sub>×2). <sup>13</sup>C-NMR (CDCl<sub>3</sub>) δ: 184.0 (C-9), 182.1 (C-10), 159.2 (C-8), 158.9 (C-1), 147.8 (C-3), 134.7 (C-14), 134.5 (C-11), 133.5 (C-6), 124.3 (C-13), 123.0 (C-12), 119.6 (C-7), 118.9 (C-5), 117.0 (C-4), 116.3 (C-2), 69.8 (OCH<sub>2</sub>×2), 64.2 (C-15), 31.9 (CH<sub>2</sub>×2), 29.6 (CH<sub>2</sub>×4), 29.4 (CH<sub>2</sub>×2), 29.3 (CH<sub>2</sub>×4), 29.1 (CH<sub>2</sub>×2), 29.0 (CH<sub>2</sub>×2), 25.9 (CH<sub>2</sub>×2), 22.6 (CH<sub>2</sub>×2), 14.1 (CH<sub>3</sub>×2).

**1,8-Di-*O*-octadecylaloe-emodin (8)** The general alkylation of **3** (100 mg, 0.37 mmol) with octadecyl iodide (700 mg, 1.8 mmol) gave **8** (103 mg, 36.0%) as yellow needles, mp 114–116 °C (after recrystallization from acetone). FAB-MS: *m/z* 775 [M+H]<sup>+</sup>. HR-MS (FAB): *m/z* Calcd for C<sub>51</sub>H<sub>83</sub>O<sub>5</sub> [M+H]<sup>+</sup>; 775.6240, Found; 775.6224. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 7.78 (1H, dd, *J*=7.6, 0.9 Hz, H-5), 7.67 (1H, brs, H-4), 7.58 (1H, t, *J*=7.6 Hz, H-6), 7.31 (1H, brs, H-2), 7.26 (1H, dd, *J*=7.6, 0.9 Hz, H-7), 4.77 (2H, s, 15-CH<sub>2</sub>OH), 4.12 and 4.11 (each 2H, t, *J*=6.4 Hz, 1- or 8-OCH<sub>2</sub>), 1.91 (4H, m, CH<sub>2</sub>×2), 1.55 (4H, m, CH<sub>2</sub>×2), 1.25 (56H, m, CH<sub>2</sub>×28), 0.88 (6H, t, *J*=7.0 Hz, CH<sub>3</sub>×2). <sup>13</sup>C-NMR (CDCl<sub>3</sub>) δ: 184.2 (C-9), 182.0 (C-10), 159.4 (C-8), 159.0 (C-1), 147.3 (C-3), 134.8 (C-14), 134.8 (C-11), 133.5 (C-6), 124.5 (C-13), 123.5 (C-12), 119.7 (C-7), 118.9 (C-5), 117.1 (C-4), 116.3 (C-2), 69.9 (OCH<sub>2</sub>×2), 64.5 (C-15), 31.9 (CH<sub>2</sub>×2), 29.8 (CH<sub>2</sub>), 29.7 (CH<sub>2</sub>), 29.5 (CH<sub>2</sub>×4), 29.4 (CH<sub>2</sub>×10), 29.2 (CH<sub>2</sub>×8), 29.1 (CH<sub>2</sub>×2), 26.0 (CH<sub>2</sub>×2), 22.7 (CH<sub>2</sub>×2), 14.1 (CH<sub>3</sub>×2).

**15-Bromochrysophanol (9)** To a solution of aloe-emodin **3** (500 mg, 1.85 mmol) in THF (100 ml), PPh<sub>3</sub> (2.65 g, 10.1 mmol) and CBr<sub>4</sub> (3.35 g, 10.1 mmol) were added and stirred at room temperature for 2 h. The reaction mixture was filtered and the filtrate was evaporated to give a residue that was subjected to column chromatography (toluene) to obtain compound **9** (505 mg, 81.9%) as a yellow solid (mp 221–223 °C). EI-MS: *m/z* 332 [M]<sup>+</sup> and 334 [M+2]<sup>+</sup>. HR-MS (EI): *m/z* Calcd for C<sub>15</sub>H<sub>9</sub>BrO<sub>4</sub> [M]<sup>+</sup>; 331.9684, Found; 331.9691. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 11.99 and 11.96 (each 1H, s, 1- or 8-OH), 7.78 (1H, dd, *J*=7.3, 1.2 Hz, H-5), 7.78 (1H, d, *J*=1.5 Hz, H-4), 7.63 (1H, t, *J*=7.3 Hz, H-6), 7.26 (1H, d, *J*=1.5 Hz, H-2), 7.25 (1H, dd, *J*=7.3, 1.2 Hz, H-7), 4.41 (2H, s, 15-CH<sub>2</sub>Br).

**15-Diethylaminochrysophanol (10)** To a solution of **9** (100 mg, 0.3 mmol) in DMF (10 ml), diethylamine (250 μl, 2.4 mmol) was added and stirred at room temperature for 12 h. The reaction mixture was poured into ice-cold water (50 ml), and then extracted with AcOEt (50 ml×3). The extracts were successively washed with 10% NaHCO<sub>3</sub> and water, and then dried over absolute MgSO<sub>4</sub> and filtered. The filtrate was evaporated to give a residue that was subjected to preparative TLC (5% MeOH in CH<sub>2</sub>Cl<sub>2</sub>) to obtain compound **10** (46.9 mg, 48.3% yield, mp 97–99 °C after recrystallization from acetone). EI-MS: *m/z* 325 [M]<sup>+</sup>. HR-MS (EI): *m/z* Calcd for C<sub>19</sub>H<sub>19</sub>NO<sub>4</sub> [M]<sup>+</sup>; 325.1313, Found; 325.1314. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 12.10 (2H, 1-, 8-OH), 7.82 (1H, dd, *J*=7.6, 1.2 Hz, H-5), 7.81 (1H, d, *J*=0.9 Hz, H-4), 7.77 (1H, t, *J*=7.6 Hz, H-6), 7.39 (1H, d, *J*=0.9 Hz, H-2), 7.28 (1H, dd, *J*=7.6, 1.2 Hz, H-7), 3.68 (2H, s, 15-CH<sub>2</sub>), 2.60 (4H, q, *J*=7.0 Hz,

$\text{NCH}_2\text{CH}_3 \times 2$ ), 1.08 (6H, t,  $J=7.0$  Hz,  $\text{NCH}_2\text{CH}_3 \times 2$ ). The  $^{13}\text{C}$ -NMR signals are listed in Table 4.

**15-(Pyrrolidin-1-yl)chrysophanol (11)** The general amination of **9** (100 mg, 0.3 mmol) with pyrrolidine (150  $\mu\text{L}$ , 1.8 mmol) gave **11** (56.2 mg, 58.0%) as yellow needles, mp 123–125 °C (after recrystallization from acetone). FAB-MS:  $m/z$  324  $[\text{M}+\text{H}]^+$ . HR-MS (FAB):  $m/z$  Calcd for  $\text{C}_{19}\text{H}_{18}\text{NO}_4$   $[\text{M}+\text{H}]^+$ ; 324.1233, Found; 324.1236.  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ )  $\delta$ : 12.08 (2H, s, 1-, 8-OH), 7.81 (1H, dd,  $J=7.6$ , 0.9 Hz, H-5), 7.80 (1H, brs, H-4), 7.77 (1H, t,  $J=7.6$  Hz, H-6), 7.35 (1H, brs, H-2), 7.30 (1H, dd,  $J=7.9$ , 0.9 Hz, H-7), 3.75 (2H, s, 15- $\text{CH}_2$ ), 2.60 (4H, m), and 1.84 (4H, m) (signals on the pyrrolidinyl group). The  $^{13}\text{C}$ -NMR signals are listed in Table 4.

**15-(Piperidin-1-yl)chrysophanol (12)** The general amination of **9** (100 mg, 0.3 mmol) with piperidine (250  $\mu\text{L}$ , 2.5 mmol) gave **12** (28.9 mg, 28.6%) as yellow needles, mp 129–131 °C (after recrystallization from acetone). FAB-MS:  $m/z$  338  $[\text{M}+\text{H}]^+$ . HR-MS (FAB):  $m/z$  Calcd for  $\text{C}_{20}\text{H}_{20}\text{N}_2\text{O}_4$   $[\text{M}+\text{H}]^+$ ; 338.1404, Found; 338.1392.  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ )  $\delta$ : 12.02 and 11.95 (each s, 1H, 1- or 8-OH), 7.74 (1H, dd,  $J=7.3$ , 1.2 Hz, H-5), 7.71 (1H, d,  $J=1.5$  Hz, H-4), 7.59 (1H, t,  $J=7.3$  Hz, H-6), 7.26 (1H, d,  $J=1.5$  Hz, H-2), 7.21 (1H, dd,  $J=7.3$ , 1.2 Hz, H-7), 3.47 (2H, s, 15- $\text{CH}_2$ ), 2.35, 1.54 and 1.38 ( $\text{CH}_2 \times 2$ ,  $\text{CH}_2 \times 2$  and  $\text{CH}_2$ , respectively, on the piperidinyl group). The  $^{13}\text{C}$ -NMR signals are listed in Table 4.

**15-(4-Methylpiperazin-1-yl)chrysophanol (13)** The general amination of **9** (100 mg, 0.3 mmol) with 1-methylpiperazine (230  $\mu\text{L}$ , 2.1 mmol) gave **13** (35.6 mg, 33.7%) as yellow needles, mp 151–153 °C (after recrystallization from acetone). FAB-MS:  $m/z$  353  $[\text{M}+\text{H}]^+$ . HR-MS (FAB):  $m/z$  Calcd for  $\text{C}_{20}\text{H}_{21}\text{N}_2\text{O}_4$   $[\text{M}+\text{H}]^+$ ; 353.1517, Found; 353.1501.  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ )  $\delta$ : 12.03 (2H, s, 1-, 8-OH), 7.82 (1H, dd,  $J=7.3$ , 1.2 Hz, H-5), 7.80 (1H, d,  $J=1.5$  Hz, H-4), 7.67 (1H, t,  $J=7.3$  Hz, H-6), 7.31 (1H, d,  $J=1.5$  Hz, H-2), 7.29 (1H, dd,  $J=7.3$ , 1.2 Hz, H-7), 3.59 (2H, s, 15- $\text{CH}_2$ ), 3.32 (8H, m,  $\text{NCH}_2 \times 4$ ), 1.25 (3H, s,  $\text{NCH}_3$ ). The  $^{13}\text{C}$ -NMR signals are listed in Table 4.

**15-(1*H*-Imidazol-1-yl)chrysophanol (14)** The general amination of **9** (100 mg, 0.3 mmol) with imidazole (102 mg, 1.5 mmol) gave **14** (18.5 mg, 19.3%) as yellow needles, mp 208–210 °C (after recrystallization from acetone). EI-MS:  $m/z$  320  $[\text{M}]^+$ . HR-MS (EI):  $m/z$  Calcd for  $\text{C}_{18}\text{H}_{12}\text{N}_2\text{O}_4$   $[\text{M}]^+$ ; 320.0801, Found; 320.0797.  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ )  $\delta$ : 12.00 (2H, s, 1-, 8-OH), 7.83 (1H, dd,  $J=7.3$ , 0.9 Hz, H-5), 7.71 (1H, t,  $J=7.3$  Hz, H-6), 7.64 (1H, brs, H-4), 7.32 (1H, dd,  $J=7.7$ , 0.9 Hz, H-7), 7.72, 7.69 and 7.54 (each 1H, proton on the imidazolyl group), 6.97 (1H, brs, H-2), 5.24 (2H, s, 15- $\text{CH}_2$ ). The  $^{13}\text{C}$ -NMR signals are listed in Table 4.

**1,8-Di-*O*-hexyl-15-*O*-tosylaloe-emodin (16)** To a solution of **6** (440 mg, 1.0 mmol) in ether (100 ml), tosylchloride (1.0 g, 5.3 mmol) and K<sub>2</sub>CO<sub>3</sub> (1.27 g, 22.6 mmol) were added and stirred at room temperature for 1 h. The reaction mixture was poured into ice-cold water (100 ml) and extracted with AcOEt (100 ml  $\times$  3). The extracts were successively washed with 5% HCl, 10% NaHCO<sub>3</sub>, brine and water, and then dried over absolute MgSO<sub>4</sub> and filtered. The filtrate was evaporated to give a residue that was subjected to column chromatography (a gradient of 0–1% acetone in toluene) to obtain compound **16** (316 mg, 53.3%). FAB-MS:  $m/z$  593  $[\text{M}+\text{H}]^+$ .  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ )  $\delta$ : 7.77 and 7.29 (each 2H, d,  $J=8.5$  Hz,  $\text{C}_6\text{H}_4$ ), 7.74 (1H, dd,  $J=7.6$ , 0.9 Hz, H-5), 7.56 (1H, t,  $J=7.6$  Hz, H-6), 7.54 (1H, d,  $J=1.5$  Hz, H-4), 7.25 (1H, dd,  $J=7.6$ , 0.9 Hz, H-7), 7.15 (1H, d,  $J=1.5$  Hz, H-2), 5.09 (2H, s, 15- $\text{CH}_2\text{O}$ ), 4.08 and 4.03 (each 2H, t,  $J=6.7$  Hz, 1- or 8- $\text{OCH}_2$ ), 2.38 (3H, s,  $\text{C}_6\text{H}_4\text{CH}_3$ ), 1.84 (4H, m,  $\text{CH}_2 \times 2$ ), 1.53 (4H, m,  $\text{CH}_2 \times 2$ ), 1.35 (8H, m,  $\text{CH}_2 \times 4$ ), 0.90 (6H, m,  $\text{CH}_3 \times 2$ ).  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ )  $\delta$ : 183.7 (C-9), 181.5 (C-10), 159.2 (C-8), 158.9 (C-1), 145.2 (CH<sub>3</sub>C<sub>6</sub>H<sub>4</sub>SO<sub>2</sub>), 139.1 (C-3), 134.9 (C-14), 134.5 (C-11), 133.6 (C-6), 132.8 (CH<sub>3</sub>C<sub>6</sub>H<sub>4</sub>SO<sub>2</sub>), 129.9 and 127.9 (CH<sub>3</sub>C<sub>6</sub>H<sub>4</sub>SO<sub>2</sub>), 124.5 (C-12), 124.3 (C-13), 119.6 (C-7), 118.7 (C-5), 118.1 (C-2), 117.7 (C-4), 70.5 (C-15), 69.9 and 69.8 (1-, 8- $\text{OCH}_2$ ), 31.5 ( $\text{CH}_2 \times 2$ ), 29.0 ( $\text{CH}_2 \times 2$ ), 25.5 ( $\text{CH}_2 \times 2$ ), 22.5 ( $\text{CH}_2 \times 2$ ), 21.6 (CH<sub>3</sub>C<sub>6</sub>H<sub>4</sub>SO<sub>2</sub>), 14.0 ( $\text{CH}_3 \times 2$ ).

**1,8-Di-*O*-hexyl-15-iodochrysophanol (15)** To a solution of compound **16** (1.0 g, 1.7 mmol) in 3-pentanone (120 ml), NaI (500 mg, 3.4 mmol) was added and stirred at room temperature for 1 h. The reaction mixture was poured into ice-cold water (100 ml) and extracted with AcOEt (100 ml  $\times$  3). The extracts were successively washed with 10% Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, brine and water, and then dried over absolute Na<sub>2</sub>SO<sub>4</sub> and filtered. The filtrate was evaporated to give a residue that was subjected to column chromatography (toluene) to obtain compound **15** (520 mg, 55.8%) as a yellow solid (mp 145–147 °C). FAB-MS:  $m/z$  549  $[\text{M}+\text{H}]^+$ . HR-MS (FAB):  $m/z$  Calcd for  $\text{C}_{27}\text{H}_{34}\text{IO}_4$   $[\text{M}+\text{H}]^+$ ; 549.1509, Found; 549.1502.  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ )  $\delta$ : 7.77 (1H, dd,  $J=7.6$ , 0.9 Hz, H-5), 7.76 (1H, d,  $J=1.5$  Hz, H-4), 7.56 (1H, t,  $J=7.6$  Hz, H-6), 7.25 (1H, dd,  $J=7.6$ , 0.9 Hz, H-7), 7.21 (1H, d,  $J=1.5$  Hz, H-2), 4.44 (2H, s, 15- $\text{CH}_2\text{I}$ ), 4.11 and 4.09 (each 2H, t,  $J=6.7$  Hz, 1- or 8- $\text{OCH}_2$ ), 1.88 (4H, m,  $\text{CH}_2 \times 2$ ), 1.54 (4H, m,  $\text{CH}_2 \times 2$ ), 1.35 (8H, m,  $\text{CH}_2 \times 4$ ), 0.90 (6H, m,

$\text{CH}_3 \times 2$ ).  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ )  $\delta$ : 183.9 (C-9), 181.5 (C-10), 159.3 (C-8), 158.9 (C-1), 145.2 (C-3), 135.2 (C-14), 134.7 (C-11), 133.5 (C-6), 124.5 (C-12), 123.9 (C-13), 119.6 (C-7), 119.3 (C-5), 118.8 (C-2), 118.6 (C-4), 69.9 and 69.8 (1-, 8- $\text{OCH}_2$ ), 31.5 ( $\text{CH}_2 \times 2$ ), 29.7 (C-15), 29.0 ( $\text{CH}_2 \times 2$ ), 25.5 ( $\text{CH}_2 \times 2$ ), 22.6 ( $\text{CH}_2 \times 2$ ), 14.0 ( $\text{CH}_3 \times 2$ ).

**15-Diethylamino-1,8-di-*O*-hexylchrysophanol (17)** To a solution of **15** (100 mg, 0.18 mmol) in DMF (10 ml), diethylamine (110 ml, 1.1 mmol) was added and stirred at room temperature for 4 h. The reaction mixture was poured into ice-cold water (50 ml), and then extracted with AcOEt (30 ml  $\times$  3). The extracts were successively washed with 10% NaHCO<sub>3</sub> and water, and then dried over absolute MgSO<sub>4</sub> and filtered. The filtrate was evaporated to give a residue that was subjected to column chromatography (a gradient of 0–3% MeOH in CHCl<sub>3</sub>) to obtain compound **17** (51.5 mg, 58.0% yield) as a yellow paste-like solid. FAB-MS:  $m/z$  494  $[\text{M}+\text{H}]^+$ .  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ )  $\delta$ : 7.72 (1H, dd,  $J=7.6$ , 0.9 Hz, H-5), 7.64 (1H, d,  $J=1.5$  Hz, H-4), 7.49 (1H, t,  $J=7.6$  Hz, H-6), 7.36 (1H, d,  $J=1.5$  Hz, H-2), 7.18 (1H, dd,  $J=7.6$ , 0.9 Hz, H-7), 4.06 and 4.04 (each 2H, t,  $J=6.7$  Hz, 1- or 8- $\text{OCH}_2$ ), 3.60 (2H, s, 15- $\text{CH}_2$ ), 2.51 (4H, q,  $J=7.0$  Hz,  $\text{NCH}_2\text{CH}_3 \times 2$ ), 1.83 (4H, m,  $\text{CH}_2 \times 2$ ), 1.49 (4H, m,  $\text{CH}_2 \times 2$ ), 1.30 (8H, m,  $\text{CH}_2 \times 4$ ), 1.00 (6H, t,  $J=7.0$  Hz,  $\text{NCH}_2\text{CH}_3 \times 2$ ), 0.84 (6H, m,  $\text{CH}_2\text{CH}_3 \times 2$ ). The  $^{13}\text{C}$ -NMR signals are listed in Table 4.

**1,8-Di-*O*-hexyl-15-(pyrrolidin-1-yl)chrysophanol (18)** The general amination of **15** (100 mg, 0.18 mmol) with pyrrolidine (92  $\mu\text{L}$ , 1.1 mmol) gave **18** (54.8 mg, 62.0%) as a yellow paste-like solid. FAB-MS:  $m/z$  492  $[\text{M}+\text{H}]^+$ .  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ )  $\delta$ : 7.75 (1H, dd,  $J=7.6$ , 1.2 Hz, H-5), 7.67 (1H, d,  $J=1.2$  Hz, H-4), 7.54 (1H, t,  $J=7.6$  Hz, H-6), 7.48 (1H, d,  $J=1.2$  Hz, H-2), 7.23 (1H, dd,  $J=7.6$ , 1.2 Hz, H-7), 4.10 and 4.08 (each 2H, t,  $J=6.7$  Hz, 1- or 8- $\text{OCH}_2$ ), 3.81 (2H, s, 15- $\text{CH}_2$ ), 2.69 (4H,  $\text{NCH}_2 \times 2$  on the pyrrolidinyl group), 1.87 (4H,  $\text{CH}_2 \times 2$  on the pyrrolidinyl group), 1.85 (4H, m,  $\text{CH}_2 \times 2$ ), 1.51 (4H, m,  $\text{CH}_2 \times 2$ ), 1.33 (8H, m,  $\text{CH}_2 \times 4$ ), 0.88 (6H, m,  $\text{CH}_3 \times 2$ ). The  $^{13}\text{C}$ -NMR signals are listed in Table 4.

**1,8-Di-*O*-hexyl-15-(piperidin-1-yl)chrysophanol (19)** The general amination of **15** (100 mg, 0.18 mmol) with piperidine (110  $\mu\text{L}$ , 1.1 mmol) gave **19** (47.2 mg, 52.0%) as yellow paste-like solids. FAB-MS:  $m/z$  506  $[\text{M}+\text{H}]^+$ .  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ )  $\delta$ : 7.76 (1H, dd,  $J=7.6$ , 0.9 Hz, H-5), 7.67 (1H, d,  $J=1.2$  Hz, H-4), 7.56 (1H, t,  $J=7.6$  Hz, H-6), 7.44 (1H, d,  $J=1.2$  Hz, H-2), 7.24 (1H, dd,  $J=7.6$ , 0.9 Hz, H-7), 4.10 and 4.08 (each 2H, t,  $J=6.7$  Hz, 1- or 8- $\text{OCH}_2$ ), 3.70 (2H, s, 15- $\text{CH}_2$ ), 2.55 (4H,  $\text{NCH}_2 \times 2$  on the piperidinyl group), 1.81 (4H, m,  $\text{CH}_2 \times 2$ ), 1.63 (4H,  $\text{CH}_2 \times 2$  on the piperidinyl group), 1.53 (4H, m,  $\text{CH}_2 \times 2$ ), 1.46 (2H,  $\text{CH}_2$  on the piperidinyl group), 1.34 (8H, m,  $\text{CH}_2 \times 4$ ), 0.89 (6H, m,  $\text{CH}_3 \times 2$ ). The  $^{13}\text{C}$ -NMR signals are listed in Table 4.

**1,8-Di-*O*-hexyl-15-(4-methylpiperazin-1-yl)chrysophanol (20)** The general amination of **15** (100 mg, 0.18 mmol) with 1-methylpiperazine (100  $\mu\text{L}$ , 0.9 mmol) gave **20** (45.2 mg, 48.3%) as a yellow paste-like solid. FAB-MS:  $m/z$  521  $[\text{M}+\text{H}]^+$ .  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ )  $\delta$ : 7.77 (1H, d,  $J=7.6$  Hz, H-5), 7.71 (1H, brs, H-4), 7.55 (1H, t,  $J=7.6$  Hz, H-6), 7.29 (1H, brs, H-2), 7.23 (1H, d,  $J=7.6$  Hz, H-7), 4.10 and 4.09 (each 2H, t,  $J=6.7$  Hz, 1- or 8- $\text{OCH}_2$ ), 3.55 (2H, s, 15- $\text{CH}_2$ ), 2.48 (8H, m,  $\text{CH}_2 \times 4$  on the piperazinyl group), 2.28 (3H,  $\text{NCH}_3$  on the piperazinyl group), 1.88 (4H,  $\text{CH}_2 \times 2$ ), 1.55 (4H, m,  $\text{CH}_2 \times 2$ ), 1.35 (8H, m,  $\text{CH}_2 \times 4$ ), 0.90 (6H, m,  $\text{CH}_3 \times 2$ ). The  $^{13}\text{C}$ -NMR signals are listed in Table 4.

**1,8-Di-*O*-hexyl-15-(1*H*-imidazol-1-yl)chrysophanol (21)** The general amination of **15** (100 mg, 0.18 mmol) with imidazole (61.2 mg, 0.9 mmol) gave **21** (58 mg, 66.5%) as yellow needles (mp 147–148 °C after recrystallization from acetone). FAB-MS:  $m/z$  489  $[\text{M}+\text{H}]^+$ . HR-MS (FAB):  $m/z$  Calcd for  $\text{C}_{30}\text{H}_{37}\text{N}_2\text{O}_4$   $[\text{M}+\text{H}]^+$ ; 489.2771, Found; 489.2771.  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ )  $\delta$ : 7.75 (1H, dd,  $J=7.6$ , 0.9 Hz, H-5), 7.61 (1H, d,  $J=1.5$  Hz, H-4), 7.57 (1H, s, a proton on the imidazolyl group), 7.56 (1H, t,  $J=7.6$  Hz, H-6), 7.25 (1H, dd,  $J=7.6$ , 0.9 Hz, H-7), 7.11 and 6.90 (each 1H, s, a proton on the imidazolyl group), 6.84 (1H, d,  $J=1.5$  Hz, H-2), 5.17 (2H, 15- $\text{CH}_2$ ), 4.09 and 3.97 (each 2H, t,  $J=6.7$  Hz, 1- or 8- $\text{OCH}_2$ ), 1.85 (4H, m,  $\text{CH}_2 \times 2$ ), 1.51 (4H, m,  $\text{CH}_2 \times 2$ ), 1.33 (8H, m,  $\text{CH}_2 \times 4$ ), 0.89 (6H, m,  $\text{CH}_3 \times 2$ ). The  $^{13}\text{C}$ -NMR signals are listed in Table 4.

**15-Thiocyanochrysophanol (22)** A solution of bromide **9** (50 mg, 0.15 mmol) in absolute DMF (10 ml) was added with KSCN (58 mg, 0.6 mmol) and stirred at 65 °C for 1 h. The reaction mixture was poured into ice-cold water (20 ml) and extracted with CH<sub>2</sub>Cl<sub>2</sub> (20 ml  $\times$  3). The extracts were washed successively with brine and water, and then dried over absolute Na<sub>2</sub>SO<sub>4</sub> and filtered. The filtrate was evaporated to give a residue that was subjected to column chromatography (a gradient of 0–2% AcOEt in toluene) to obtain compound **22** (25 mg, 53.5%) as solid (mp 192–194 °C). EI-MS:  $m/z$  311  $[\text{M}]^+$ . HR-MS (FAB):  $m/z$  Calcd for  $\text{C}_{16}\text{H}_9\text{NO}_4\text{S}$   $[\text{M}]^+$ ; 311.0252, Found; 311.0262.  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ )  $\delta$ : 12.02 (1H, s, OH), 11.93

Table 4.  $^{13}\text{C}$ -NMR Spectral Data of Various 15-Aminoderivatives **10**—**14** and **17**—**21**<sup>a)</sup>

	10	11	12	13	14	17	18	19	20	21
C-1	162.5	162.4	161.4	162.5	162.7	158.8	158.8	158.9	158.8	158.9
C-2	114.7	114.7	113.6	114.7	115.6	118.8	118.8	119.4	119.0	117.1
C-3	151.4	150.3	149.4	150.0	167.7	134.9	133.4	135.0	145.0	142.0
C-4	115.9	115.9	114.9	115.8	115.7	118.7	118.7	118.7	118.7	117.0
C-5	120.0	120.0	118.9	120.0	120.3	119.5	119.6	119.6	119.5	118.8
C-6	133.4	133.5	132.2	133.4	132.5	133.3	129.4	133.4	133.3	133.6
C-7	120.5	120.5	119.7	120.6	120.3	119.6	119.8	119.8	119.5	119.7
C-8	162.8	162.9	161.7	162.8	163.1	159.2	159.3	159.2	159.1	159.7
C-9	192.6	192.6	191.6	192.6	192.5	184.5	184.3	184.4	184.4	183.8
C-10	181.9	181.8	180.9	181.9	181.3	180.2	181.9	181.9	181.9	181.4
C-11	133.7	133.7	132.7	133.7	133.4	134.9	134.7	134.8	134.8	135.2
C-12	124.6	124.6	123.5	124.6	125.0	124.7	124.6	124.6	124.6	124.3
C-13	124.0	124.0	123.1	124.0	124.9	123.4	123.7	123.8	123.5	124.2
C-14	137.0	137.1	136.0	137.1	137.5	134.5	134.6	134.5	134.6	134.5
C-15	57.3	60.0	62.1	62.2	68.2	57.4	59.7	62.4	62.4	50.4
C-2'	47.2	54.2	53.6	53.1	137.5	46.9	53.9	54.0	53.0	137.5
C-3'	11.8	23.6	24.9	55.0	—	11.6	23.4	25.0	55.0	—
C-4'	47.2	23.6	23.1	—	130.9	46.9	23.3	23.7	—	130.3
C-5'	11.8	54.2	24.9	55.0	120.3	—	11.6	25.0	55.0	119.2
C-6'	—	—	53.6	53.1	—	—	—	54.0	53.0	—
C-7'	—	—	—	45.9	—	—	—	—	45.9	—
OCH <sub>2</sub>	—	—	—	—	—	69.8	69.9	69.9	69.8	69.9
						69.7	69.8	69.8	69.7	69.8
CH <sub>2</sub>	—	—	—	—	—	31.5×2 <sup>b)</sup>	31.5×2	31.6	31.5×2	31.5×2
						29.0×2	29.0×2	31.5	29.0×2	29.0
						25.5	25.5×2	29.0×2	25.5×2	28.9
						25.6	22.5×2	25.5×2	22.5×2	25.5×2
						22.5×2	—	22.6	—	22.5×2
						—	—	22.5	—	—
CH <sub>3</sub>	—	—	—	—	—	14.0×2	14.0×2	14.0×2	14.0×2	14.0×2

a) Spectra were obtained in CDCl<sub>3</sub>. b) 31.5×2: two carbon signals overlapped at  $\delta$  31.5 and others in a similar manner.

(1H, s, OH), 7.79 (1H, dd,  $J=7.3$ , 1.2 Hz, H-5), 7.76 (1H, d,  $J=1.5$  Hz, H-4), 7.65 (1H, t,  $J=7.3$  Hz, H-6), 7.27 (1H, dd,  $J=7.3$ , 1.2 Hz, H-7), 7.25 (1H, d,  $J=1.5$  Hz, H-2), 4.11 (2H, 15-CH<sub>2</sub>).

**15-Selenocyanochrysophanol (23)** A solution of bromide **9** (50 mg, 0.15 mmol) in absolute DMF (10 ml) was added with KSeCN (87 mg, 0.6 mmol) and stirred at room temperature for 1 h. The reaction mixture was poured into ice-cold water (20 ml) and extracted with CH<sub>2</sub>Cl<sub>2</sub> (20 ml×3). The extracts were washed successively with brine and water, and then dried over absolute Na<sub>2</sub>SO<sub>4</sub> and filtered. The filtrate was evaporated to give a residue which was subjected to column chromatography (a gradient of 0—2% AcOEt in toluene) to obtain compound **23** (35 mg, 65.1%) as a yellow solid (mp 178—181 °C). EI-MS:  $m/z$  358 [M]<sup>+</sup>. HR-MS (EI):  $m/z$  Calcd for C<sub>16</sub>H<sub>9</sub>NO<sub>4</sub>Se [M]<sup>+</sup>: 358.9720, Found; 358.9697. <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$ : 12.09 (1H, s, OH), 12.02 (1H, s, OH), 7.85 (1H, dd,  $J=7.3$ , 1.2 Hz, H-5), 7.82 (1H, d,  $J=1.5$  Hz, H-4), 7.72 (1H, t,  $J=7.3$  Hz, H-6), 7.33 (1H, dd,  $J=7.3$ , 1.2 Hz, H-7), 7.30 (1H, d,  $J=1.5$  Hz, H-2), 4.28 (2H, 15-CH<sub>2</sub>).

**15-Bromo-1,8-di-*O*-hexylchrysophanol (24)** To a solution of **6** (900 mg, 2.05 mmol) in ether (100 ml), PPh<sub>3</sub> (1.8 g, 6.8 mmol) and CBr<sub>4</sub> (2.24 g, 6.8 mmol) were added and stirred at room temperature for 12 h. The reaction mixture was filtered. The filtrate was evaporated to give a residue that was subjected to column chromatography (a gradient of 0—3% AcOEt in toluene) to obtain compound **24** (850 mg, 83.0%) as a yellow solid (mp 129—131 °C). EI-MS:  $m/z$  501 [M]<sup>+</sup> and 503 [M+2]<sup>+</sup>. HR-MS (EI):  $m/z$  Calcd for C<sub>27</sub>H<sub>33</sub>BrO<sub>4</sub> [M]<sup>+</sup>: 501.1562, Found; 501.1618. <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$ : 7.80 (1H, brs, H-4), 7.79 (1H, dd,  $J=7.6$ , 0.9 Hz, H-5), 7.59 (1H, t,  $J=7.6$  Hz, H-6), 7.27 (1H, dd,  $J=7.6$ , 0.9 Hz, H-7), 7.28 (1H, brs, H-2), 4.50 (2H, s, 15-CH<sub>2</sub>Br), 4.14 and 4.12 (each 2H, d,  $J=6.7$  Hz, 1- or 8-OCH<sub>2</sub>), 1.91 (4H, m, CH<sub>2</sub>×2), 1.57 (4H, m, CH<sub>2</sub>×2), 1.38 (8H, m, CH<sub>2</sub>×4), 0.93 (6H, m, CH<sub>3</sub>×2). <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$ : 183.8 (C-9), 181.5 (C-10), 159.3 (C-8), 158.9 (C-1), 143.3 (C-3), 135.1 (C-14), 134.6 (C-11), 133.6 (C-6), 124.4 (C-12), 124.2 (C-13), 119.6 (C-7), 119.5 (C-5), 118.9 (C-2), 118.8 (C-4), 69.9 and 69.8 (1- and 8-O-CH<sub>2</sub>), 31.9 (C-15), 31.5 (CH<sub>2</sub>×2), 29.0 (CH<sub>2</sub>×2), 25.5 (CH<sub>2</sub>×2), 22.5 (CH<sub>2</sub>×2), 14.0 (CH<sub>3</sub>×2).

**1,8-Di-*O*-hexyl-15-thiocyanochrysophanol (25)** A solution of bromide **24** (50 mg, 0.10 mmol) in absolute DMF (10 ml) was added with KSCN (39 mg, 0.40 mmol) and stirred at 65 °C for 2 h. The reaction mixture was

poured into ice-cold water (20 ml) and extracted with CH<sub>2</sub>Cl<sub>2</sub> (20 ml×3). The extracts were washed successively with brine and water, and then dried over absolute Na<sub>2</sub>SO<sub>4</sub> and filtered. The filtrate was evaporated to a residue that was recrystallized from ethanol to give compound **25** (37 mg, 76.6%) as yellow needles (mp 134—136 °C). FAB-MS:  $m/z$  480 [M+H]<sup>+</sup>. HR-MS (FAB):  $m/z$  Calcd for C<sub>28</sub>H<sub>34</sub>NO<sub>4</sub>S [M+H]<sup>+</sup>: 480.2209, Found; 480.2213. <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$ : 7.80 (1H, dd,  $J=7.6$ , 0.9 Hz, H-5), 7.78 (1H, d,  $J=1.5$  Hz, H-4), 7.60 (1H, t,  $J=7.6$  Hz, H-6), 7.29 (1H, dd,  $J=7.6$ , 0.9 Hz, H-7), 7.26 (1H, d,  $J=1.5$  Hz, H-2), 4.19 (2H, 15-CH<sub>2</sub>), 4.16 and 4.13 (each 2H, t,  $J=6.7$  Hz, 1- or 8-OCH<sub>2</sub>), 1.92 (4H, m, CH<sub>2</sub>×2), 1.57 (4H, m, CH<sub>2</sub>×2), 1.38 (8H, m, CH<sub>2</sub>×4), 0.93 (6H, m, CH<sub>3</sub>×2).

**1,8-Di-*O*-hexyl-15-selenocyanochrysophanol (26)** A solution of bromide **24** (50 mg, 0.10 mmol) in absolute DMF (10 ml) was added with KSeCN (58 mg, 0.40 mmol) and stirred at 65 °C for 1 h. The reaction mixture was poured into ice-cold water (20 ml) and extracted with CH<sub>2</sub>Cl<sub>2</sub> (20 ml×3). The extracts were washed successively with brine and water, dried over absolute Na<sub>2</sub>SO<sub>4</sub>, and then filtered. The filtrate was evaporated to a residue that was subjected to column chromatography (a gradient of 0—6% AcOEt in toluene) to obtain compound **26** (45 mg, 86.2%) as a yellow powder (mp 130—133 °C). FAB-MS:  $m/z$  528 [M+H]<sup>+</sup>. HR-MS (FAB):  $m/z$  Calcd for C<sub>28</sub>H<sub>34</sub>NO<sub>4</sub>Se [M+H]<sup>+</sup>: 528.1653, Found; 528.1656. <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$ : 7.79 (1H, dd,  $J=7.6$ , 0.9 Hz, H-5), 7.76 (1H, d,  $J=1.8$  Hz, H-4), 7.59 (1H, t,  $J=7.6$  Hz, H-6), 7.28 (1H, dd,  $J=7.6$ , 0.9 Hz, H-7), 7.26 (1H, d,  $J=1.8$  Hz, H-2), 4.31 (2H, 15-CH<sub>2</sub>), 4.15 and 4.12 (each 2H, t,  $J=6.7$  Hz, 1- or 8-OCH<sub>2</sub>), 1.91 (4H, m, CH<sub>2</sub>×2), 1.58 (4H, m, CH<sub>2</sub>×2), 1.37 (8H, CH<sub>2</sub>×4), 0.92 (6H, m, CH<sub>3</sub>×2).

**Cytotoxic Effects. Cell Lines and Culture** The human colorectal carcinoma cell line (HCT 116, ATCC No. CCL-247) and human hepatoma cell line (Hep G2 No. RCB0459) were purchased from Dainippon Pharmaceutical Co., Ltd. (Osaka, Japan) and RIKEN Cell Bank (Tsukuba, Japan), respectively. Dulbecco's modified Eagle's medium (DMEM), McCoy's 5A medium, fetal bovine serum (FBS) and penicillin-streptomycin mixture (100 U/ml penicillin and 100  $\mu$ g/ml streptomycin) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), Sigma (MO, U.S.A.), Biosource International (CA, U.S.A.) and Bio Whittaker (ND, U.S.A.), respectively. The HCT 116 cells were maintained in McCoy's 5A medium and

Hep G2 cells were cultured in DMEM. Each medium was supplemented with 10% FBS and a penicillin–streptomycin mixture at 37 °C in a humidified atmosphere containing 5% CO<sub>2</sub> in air.

**Assay Procedure** Aliquots (200  $\mu$ l) of  $5 \times 10^3$  cells per ml of HCT 116 and Hep G2 cells were seeded in 96 well flat-bottomed plates (Microtest™ Tissue Culture Plate, 96 Well, Flat Bottom with Low Evaporation Ltd., Falcon, NJ, U.S.A.), and they were incubated in a medium containing 10% FBS and a penicillin–streptomycin mixture at 37 °C in a humidified atmosphere of 5% CO<sub>2</sub> for 24 h. The test drugs were dissolved in dimethyl sulfoxide (DMSO). The incubation medium was replaced with each test medium giving a final concentration of 0.1–500  $\mu$ mol/l of test compounds and no drug in 2  $\mu$ l DMSO over 2 d. The ability of the drug to inhibit cellular growth was determined by performing the MTT assay.<sup>15)</sup> The cytotoxic effects of the test drugs were determined as previously described.<sup>1)</sup> Each experiment was performed in duplicate wells, and all the experiments involving a control (DMSO only) and the drug treatments were performed separately three to five times. The data represent mean  $\pm$  S.D. values.

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